Lysostaphin

Week 5

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

After the 3A assembly of pSB1K3-T7-Lys, the plasmids were purified and transformed into BL21 cells. This was followed by colony picking and PCR to run on the gel. The first trial of overhang PCR with BamHI was attempted, in order to add the linker-tag sequence.

1 Plasmid extraction of pSB1K3-T7-Lys and Lys Glycerol Stock

Responsible

Oscar He and Ellinor Lindholm

Protocols used

Plasmid Extraction

Experimental Setup

Sample T7+Lys and Glycerol Stock of Lysostaphin were extracted
The extracted sample was separated into five samples labelled as followed:

1 T7+Lys 1
2 T7+Lys 2
3 T7+Lys 3
4 Glycerol Lys 1
5 Glycerol Lys 2

Results and Conclusions

Table 1: Concentrations of extracted plasmid DNA measured using NanoDrop at 280 nm.

Sample	Concentration [ng/μl]
1 T7+Lys 1	23.6
2 T7+Lys 2	33.9
3 T7+Lys 3	32.2
4 Glycerol Lys 1	91.5
5 Glycerol Lys 2	65.5

2 Transformation of pSB1K3-T7-Lys into BL21(DE3)

Responsible

Reskandi Rudjito and Bethel Tesfai Embaie

Protocols used

Transformation

Modifications and comments to protocols

Kanamycin (plates labelled red) were used
Heat shock for 15 seconds only for BL21(DE3) cell transformation

Experimental Setup

Table 2: Volumes of the liquid cultures used for plasmid extraction. GS - Glycerol stock, HQ - Headquarters.

Plate	Transformed cell component
1	BL21 Lys+T7
2	BL21 Lys+T7
3	BL21 negative control
4	BL21 no antibiotic

Results and Conclusions

Numerous colonies grew on both transformed plates 1 and 2, as well as control plate 3. Three colonies were picked from each plate for colony PCR.

Table 3: The colonies picked were labeled as shown below:

Plate	Colony
1	Lys 1.1
	Lys 1.2
	Lys 1.3
2	Lys 2.1
	Lys 2.2
	Lys 2.3

3 Colony PCR of pSB1K3-T7-Lys

Responsible

Reskandi Rudjito,Bethel Tesfai Embaie and Shuangjia Xue

Protocols used

PCR Amplification (Q5)

Experimental Set Up

The colony PCR reaction was performed on the colonies picked from transformed BL21 cells of pSB1K3-T7-Lys. The reaction composition and condition are described in the tables below.

Table 4: Colony PCR reaction mix (total volume 12 μl).

Component	Volume [μl]	Final Concentration
5X Q5 Reaction Buffer	2.5	1X
10 mM dNTPs	0.25	0.5 M
10 M Forward Primer (VF2)	1	0.5 M
10 M Reverse Primer (VR)	1	0.5 M
Template DNA	0	<1 ng
Q5 High-Fidelity DNA Polymerase	0.125	0.02 U/l
Nuclease-Free Water	7.625	-

Table 5: PCR conditions of Q5 Polymerase (denaturation, annealing, extension 30 cycles).

Step	Temperature [°C]	Time [seconds]
Initial Denaturation	98	30
Denaturation	98	10
Annealing	52	30
Extension	72	20
Final Extension	72	120
Hold	4	-

Results and Conclusions

We ran a gel of the colony PCR products and only Lys 1.2 showed a positive band.

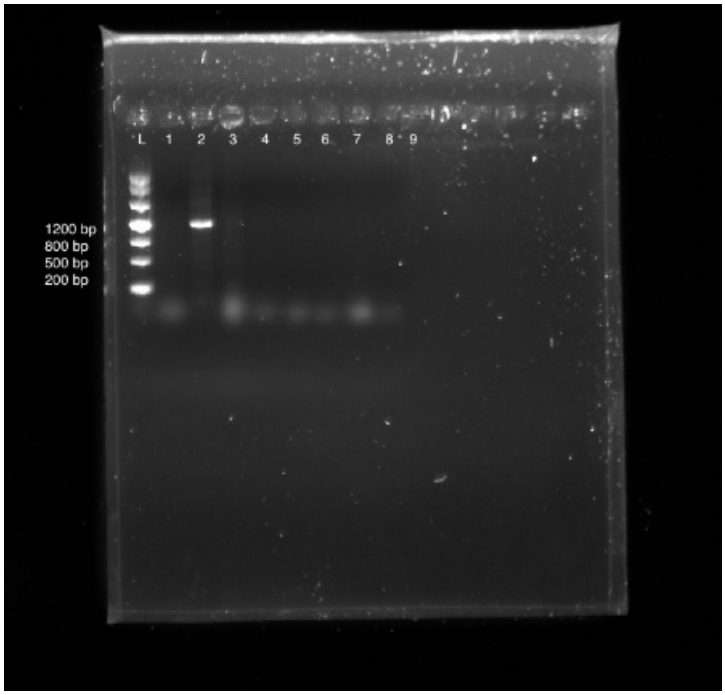

Figure 1: Colony PCR product of pSB1K3-T7-Lys. As previously labelled colonies:Lys 1.1, Lys 1.2, Lys 1.3, Lys 2.1, Lys 2.2, Lys 2.3, Lys 2.4, negative control

Discussion and Troubleshooting

No bands were present in all the colonies picked, but Lys 1.2. This may have been due to unsuccessful 3A assembly with T7 or a problem with the colony PCR performed after transformation.

4 Overhang PCR of pSB1K3-T7-Lys

Responsible

Betty Tesfai, Reskandi Rudjito, Shuangjia Xue

Protocols used

PCR Amplification (Q5)

Modifications to protocols

Two Esp plasmids were run in parallel which is why the Master Mix is calculated for more samples than represented here.

Experimental Set Up

This experiment was performed in order to add a BamHI restriction site to the 3'end of Lys, which will be needed to add the Linker-Tag sequence.
All three colonies from Lys were run here with two different PCR programs, where the annealing temperature was either 52C or 58C to optimise primer binding. Primers were always used at a concentration of 10 μM

Table 6: Experimental Set Up

Sample	**Component**
Overhang Lys1	VF2_F + Lys_R
Overhang Lys2	VF2_F + Lys_R
Overhang Lys3	VF2_F + Lys_R
Lys cross over (x)	Lys template + EB primer
EB cross over (x)	EB template + Lys primer
Lys pos. control	VF2_F + VF2_R
neg. control	no DNA
Lys plasmid	no PCR reaction

Table 7: PCR Programs

Step	**Temperature [°C]**	**Time [seconds]**
Initial Denaturation	98	30
	98	10
Main Part: 30 cycle	52 or 58	30
	72	20
Final Extension	72	2
Hold	4	-

Results and Conclusions

Table 8: PCR 52°C

Well	Sample	Description
1	Ladder	
2	Overhang Lys1	Faint band below 1000 bp
3	Overhang Lys2	Faint band below 1000 bp
4	Overhang Lys3	Faint band below 1000 bp
5	Lys pos. control	strong PCR product
6	Lys plasmid	faint band at 2000-3000 bp
11	Negative control	smear

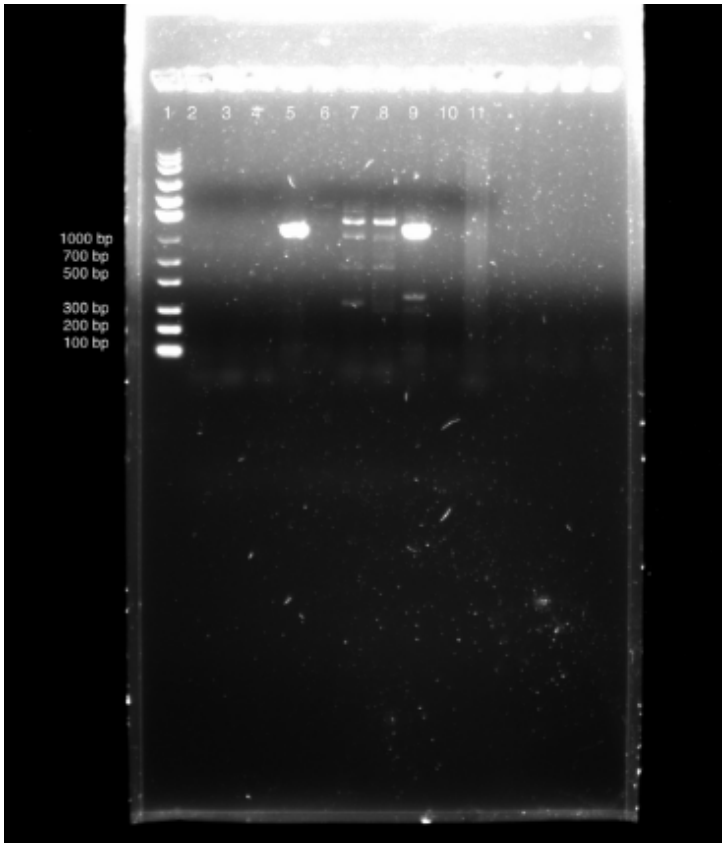

Figure 2: Analysis of overhang PCR with 52C annealing temperature

Table 9: PCR 58°C

Well	**Sample**	**Description**
1	Ladder	
2	Overhang Lys1	no product
3	Overhang Lys2	no product
4	Lys pos. control	PCR product of 1000 bp
5	Lys cross over	no product

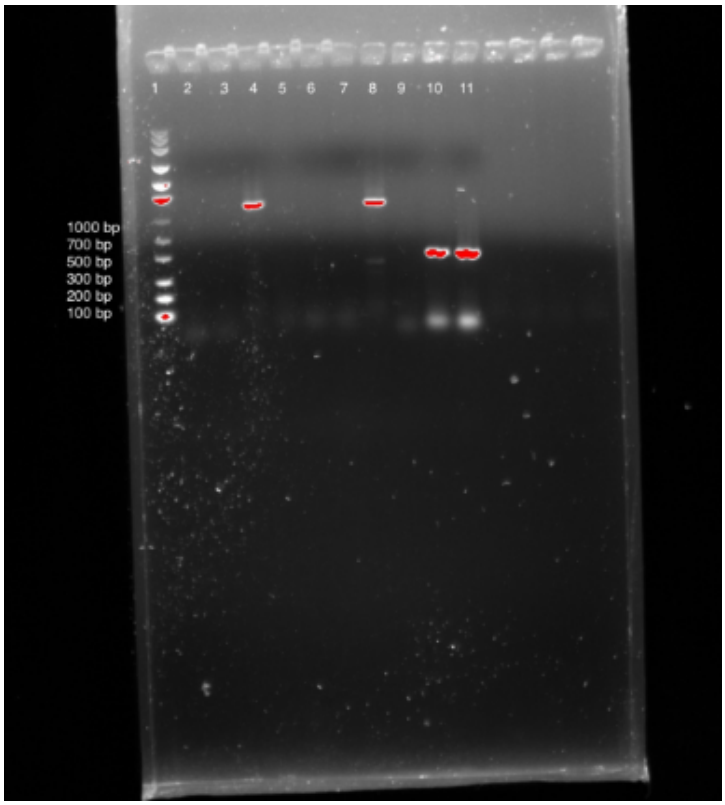

Figure 3: Analysis of overhang PCR with 58C annealing temperature

Discussion and Troubleshooting

The PCR seems to have been largely unsuccessful with PCR products that were either unspecific (at 52C annealing temperature) or simply not present (58C). Alongside the annealing temperature, further components of the PCR such as primer and MgCl concentration can be optimised. Additionally, it could be beneficial to use Taq polymerase again, which has been used more extensively in this lab than the Q5 Master Mix.